UNIVERSITY OF WISCONSIN

DEPARTMENT OF PHYSICS

X-Ray Spectroscopy and the Small Angle Scattering of X-Rays

A Final Report on Research Conducted Under Contract with the Office of Naval Research, Contract N7 onr-28503 (NR 017-605)

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I - Introduction

The contract whose results are here summarized ran from June 15, 1948 to August 31, 1953. Actual research, under the contract, began in October 1948 using facilities already built up with the assistance of the University of Wisconsin and the Wisconsin Alumni Research Foundation. This assistance has continued and has provided most of the greatly improved and expanded permanent research facilities now available. The contract has made important contributions of equipment and supplies and provided the major portion of the salary budget.

We have used x-ray spectroscopic techniques to determine electron band structures in solids and also excited electronic levels of molecules in condensed systems. The latter furnish information about bonding orbitals and, indirectly, about the symmetry of nearest neighbors around the absorbing atom.

Small angle scattering is a most useful tool in determining the size and shape of colloidal particles or in the analysis of the very large Bragg spacings which sometimes occur in organic crystals. Our principal effort has been applied to the measurement of the size, shape and hydration of protein and virus molecules in solution.

An interest in the quite different fields of solid state and biophysics originally developed at this laboratory because almost identical x-ray techniques could be used in each. The double crystal spectrometer is an excellent monochromator for spectroscopic use and also for some purposes, a very good collimator and analyser in small angle scattering experiments. It was soon found that new instrumentation was necessary for the best work in small angle scattering. Such instrumentation was developed and the new fields opened up seemed so interesting and easily exploited that spectroscopy became a minor interest.

In addition to the above fields the contract has supported some purely theoretical solid state investigations by D. L. Dexter and an investigation of the x-ray scattering from liquid helium I and II by A. G. Tweet. Dr. Tweet's measurements were confined to small angles because of the equipment available but an extension to larger angles is being planned.

Very little use has been made of technical reports under this contract. The advantages of quick publication and detailed discussion which they afford are of less compelling importance in a program whose efforts are duplicated at only a very few other laboratories. It is felt that too many contract and technical reports of limited circulation are a danger unless the important results are also reported in the regular literature.

If they are so reported, the writing of a technical report often represents an expenditure of time which might better be used in pushing the research. The papers listed under "ONR Supported Publications" at the end of this report will present a complete record of the contract research. All but one will be available in the standard journals. The list does not include references to abstracts of papers presented before scientific societies.

II X-Ray Spectroscopy

A. Instrumentation

At the beginning of the contract a double calcite crystal spectrometer was being used. It had been constructed in 1942 at this laboratory and was equipped with a vacuum jacket for work between 2 and 5 Å. Such an instrument furnishes excellent resolution and fair intensity. Geiger counters are used for detection.

In 1949-50 Mr. George Mitchell built a curved crystal vacuum spectrometer in the hope of increasing the available intensity. This project was eventually abandoned and Mr. Mitchell completed his work on the double crystal spectrometer. Our major difficulty was in obtaining sufficiently good crystals. It became apparent that a great deal of effort would have to be spent in carefully grinding and bending crystals. Curved crystal spectrometers are in use at several laboratories, but the resolution even under the best conditions is not as good as that which can be very easily obtained with a double crystal spectrometer. The intensity advantages of the curved crystal are appreciable, but our experience with rotating anode x-ray tubes, which are quite simple and reliable, indicates that the intensity problem may sometimes be more easily solved by increasing the source strength.

B. Results

By 1949 our application of x-ray spectroscopy was largely confined to problems of chemical bonding. We had observed earlier that the K absorption edge structure of the absorbing atom or ion depended strongly on its chemical bonding. In the simpler ionic compounds the structure can be understood and the state of ionization predicted from the position of the strongest absorption line which in elements of the first transition series corresponds to a $1s \rightarrow 4p$ transition. In some, but not all, covalent compounds there is found an absorption line at a position indicating that the absorbing atom is neutral. This, of course, is not unexpected in a covalent compound. However, the existence of the absorption line also implies that not all the 4p orbital has been used in bonding and thus may be used to distinguish between tetrahedral sp^3 and octahedral

d^2sp^3 bonds which make full use of the 4p orbital and square dsp^2 bonds which leave some 4p orbital available for absorption. Mr. Mitchell[10] has run a number of nickel covalent complexes, some of square and some of tetrahedral symmetry and has summarized the evidence available in the literature on other elements of the first transition series. The interpretation outlined above appears to be correct without exception. Thus x-ray spectroscopy may sometimes be of considerable use in determining bonding orbitals.

Mr. Shurman[18] extended measurements to several cobalt compounds, to oxides of vanadium and copper and to a series phthalocyanines. Again the x-ray measurements were in agreement with chemical expectation. V_2O_5 gave evidence of considerable covalent bonding.

C. Madison Conference, October 23-25, 1950

This Conference which was supported both by the Office of Naval Research and the Wisconsin Alumni Research Foundation attracted about fifty people to Madison including Professor Mott of Bristol, Professor Cauchois of Paris, Dr. Sandstrom of Uppsala, Dr. Kiestra of Groningen and representatives of all the active research groups in the United States.

The papers[5] have been published by ONR and are available as Report No PB 111027 from the Office of Technical Services, U.S. Dept. of Commerce, Washington 25, D.C.

Although many hundreds of papers have been published in the general area of x-ray spectroscopy and its applications to solid state problems, this Conference was the first to bring together a large fraction of those actively working in the field.

III Small Angle Scattering

A. Instrumentation

Our first experiments in small angle scattering made use of the double calcite crystal spectrometer with the crystals in the parallel position. The scattering specimen is placed between the two crystals and the scattered radiation analyzed by rotating the second crystal. Because of a fairly high background scattering from the crystal surfaces this method is applicable only to strong scatters such as dry colloidal particles in air or biological specimens such as collagen having a regular periodicity[11].

Ritland and Kaesberg[2] developed a slit collimator which avoided the high background of the double crystal

method and permitted measurements on dilute solutions of proteins and viruses which are quite weak scatterers. The collimator consists of two slits thirty to fifty centimeters apart. These collimate the beam and illuminate the specimen which is placed fifteen or twenty-five centimeters beyond the second slit. A second pair of slits with the same separation as the first is placed on an arm which may rotate about an axis through the specimen. These analyzing slits may be set at any angle, up to about five degrees, with respect to the incident beam. With the analyzing slits slightly away from parallelism with the collimator only a small region about the specimen is simultaneously illuminated by the collimator and seen by the analyzer. Backgrounds may be less than 10^{-7} of the straight through intensity. Scattering chambers of this design have been used for most of the work done under this contract.

Some experiments [1,2] were done with a three crystal analyzer. This device retains the very high angular resolution of the double crystal instrument but may lower backgrounds by as much as a factor of ten. However, it does not begin to compete with the slit collimator when very low backgrounds are a consideration.

Within the last year a quite different type of scattering chamber has been developed. This was done in order to avoid the troublesome slit corrections which must be applied to data taken with a slit collimator. With slits a rather considerable range of scattering angles, about the nominal setting of the instrument, is admitted. The new instrument uses pinhole collimation which, of course, greatly reduces the x-ray flux hitting the sample. Some of this is regained by using a circular pick-up slit which admits all the radiation scattered at a given angle by the specimen. The pick-up slit slides back and forth on a precision ground way with the center of the circular slit always on the axis of the collimating pinholes. The scattering angle is varied in this manner. This apparatus, which will be described by Rothwell, Leonard and Beeman[17], has proved highly successful in a number of scattering experiments.

The x-ray source in all except the earlier experiments has been a continuously cooled rotating anode x-ray tube ordinarily operated between 2400 and 3000 watts input to the target. Without this equipment most of the experiments on scattering from solutions of proteins and viruses would have been impossible. The anode is a hollow copper cylinder about 4 inches in diameter and 1.5 inches high. It is mounted on a hollow vertical steel which is driven at 900 R.P.M. by an electric motor outside the vacuum. The center of the shaft carries the cooling water to and from the anode. A single linear O-ring provides the vacuum seal around the steel shaft. The O-ring is kept well saturated

with Octoil and quite satisfactory lifetimes of between 200 and 400 hours may be obtained. The design and construction of the x-ray tube will be described in reference 17.

For the detection of the scattered photons we use argon filled geiger counters from a commercial source. About a year ago a proportional counter of a standard design was completed and when used with a single channel pulse height discriminator has proved somewhat more efficient than a geiger counter with Ross filters. In some experiments where scattering must be separated from fluorescent x-rays the proportional counter is a necessity.

B. Results

1. Dow Latex Spheres

These are spheres of polystyrene latex about 2700 Å in diameter which are widely used in electron microscopy as an internal linear standard. Using the slit collimator previously described with very narrow slits we were able to measure between 15 and 20 of the subsidiary maxima and minima of the scattering curve. This furnishes an accurate diameter for the spheres which Leonard et al[9] place at 2730 Å. Electron microscopists had previously used 2590 Å. The difference is important in some applications.

2. Multiple Scattering

Dexter[1] calculated the effect of multiple scattering on the observed curves for spherical particles. The results were applied to the particle size determination of carbon blacks. The method is much more accurate than the use of single particle scattering functions. This is partially because it permits better averaging over the particle size distribution which is almost always present in artificially produced colloids.

3. Smaller Proteins

Our first efforts in this field were due to Ritland and Kaesberg[3]. The radii of gyration of lysozyme, β-lactoglobulin, ovalbumin, bovine hemoglobin, and bovine serum albumin were measured using slit collimation and a stationary anode x-ray tube. Because of intensity limitations the results reported in ref. 3 must be considered as preliminary. A discussion of possible applications of the method was given. These include the determination of size, shape, hydration and average electron density of proteins and viruses. In principle all the results of light scattering can be obtained by x-ray scattering plus the important information contained in the shape of the scattering curve which is not obtainable from light scattering. In practice, light scattering measurements are more convenient to make.

After the construction of the rotating anode and an

improved slit collimator attention was diverted to the spherical plant viruses until Anderegg[16] undertook a detailed study of serum albumin and serum albumin mercury dimer. His work showed the necessity for careful treatment of interparticle interference effects and, therefore, for measurements at low concentrations. This is an inconvenience but not necessarily a misfortune since a great deal of information can be obtained from a study of the interparticle interference effects. Anderegg obtained a radius of gyration of 30Å for bovine serum albumin and 31Å for human serum albumin. Human serum albumin mercury dimer has a radius of gyration of 37Å and the distance between the centers of the monomer units in the dimer is 41Å. These results when taken in conjunction with other physical parameters of the molecule indicate an oblate or triaxial structure with appreciable internal hydration. This is not in agreement with current beliefs.

Rothwell[20] has run bovine serum albumin and bovine hemoglobin on the recently completed pinhole scattering device. His results on serum albumin are in excellent agreement with those of Anderegg. Rothwell studied the molecule over a wide range of concentrations and ionic strengths and at pH's from about 3.2 to 6.0. There was no evidence for a change of size or shape of the molecule in this pH range. Hemoglobin has a radius of gyration of about 26Å. Its axial ratio is around 1.3 in agreement with single crystal expectations. A first subsidiary maximum is clearly seen in the hemoglobin scattering curve. No such maximum is observed with serum albumin indicating that it deviates from a sphere considerably more than does hemoglobin.

Kaesberg[15] has run x-ray scattering curves on bovine fibrinogen using the slit collimator. The molecule is prolate with a very large axial ratio. From the x-ray data the axial ratio is about seven.

4. Spherical Viruses

There exist a number of spherical plant viruses which are particularly easy to investigate with small angle scattering since the subsidiary maxima and minima of the spherical scattering function furnish very precise information on size. Leonard[7,12] and Schmidt[14] have had primary responsibility for this work. Southern bean mosaic virus, tobacco necrosis virus and tomato bushy stunt virus were investigated by Leonard and turnip yellow mosaic virus by Schmidt. The hydrated viruses in dilute solution have diameters between 280 and 300Å. The dry viruses have calculated diameters much less than this, therefore, the x-ray measurements provide clear evidence for the internal hydration of these molecules. In the case of turnip yellow mosaic virus the hollow sphere model of the protein particle, first proposed by Markham, was confirmed.

In all cases interparticle interference effects were observed for solutions not at the isoelectric point. These indicated a collision diameter greater than the molecular diameter and thus the existence of repulsive forces between molecules.

IV Other Research

Dexter, either alone[8], or in collaboration with Sachs[4], has done two calculations on solid state problems. Neither of these was directly connected with the principal interests of the contract, but were important in broadening Dr. Dexter's experience in solid state theory which was his main interest.

Dexter[6] also did a short calculation on the fluorescence yield of argon. This helped to clear up a disagreement between two conflicting measurements in the literature and illustrated an interesting method of calculating fluorescence yields.

Tweet[13] measured the small angle scattering, using slit collimation, from liquid helium I and II in the temperature range 1.5°K to 4.2°K. As expected the scattering followed closely the thermodynamic relationship with the isothermal compressibility. At the lowest temperature the observed scattering was somewhat too great and at 4.2° there was an appreciable decrease in the scattering between 0° and 5° scattering. This is similar to the scattering behaviour of gases near their critical points.

Concurrently with the experimental work on scattering from proteins and viruses we have calculated a number of scattering functions for particles of various shapes, both with pinhole and slit scattering geometries. These will be summarized by Anderegg, Schmidt and Dexter[19].

V Conclusions and Recommendations

There remain a number of excellent problems in solid state spectroscopy. In fact, it has been suggested that most of the excellent problems remain. However, it appears that these are best done at longer wavelengths where better energy resolution is available. It also appears that a small research group cannot effectively do long wavelength solid state spectroscopy as one of several interests. The experimental techniques are difficult and the equipment not well adopted to more than the one pursuit.

We are strongly convinced of the importance of the general field of diffuse x-ray scattering from solutions and other non-crystalline macromolecular preparations. Even the minimum program of determining radii of gyration furnishes a parameter which is a very useful addition to the commonly measured macromolecular constants. The data

are vastly easier to interpret than the data of x-ray crystallography although, of course, the ultimate possibilities are not so great. The necessary equipment is not available commercially but is relatively easy to assemble if good shop facilities are at hand.

We believe that other laboratories should undertake programs in this field. In only a very few places have there been continued efforts to make these measurements with the necessary precision. The field is quite new and the results badly in need of cross checking from several independent sources.

Finally we would like to recommend to other x-ray laboratories in this country their serious consideration of the rotating anode x-ray tube. There are many experiments, particularly in the measurement of diffusely scattered x-rays, which are borderline, because of low intensity, with a stationary anode. An increase of a factor of four or five in intensity may speed up data taking by much more than this by lifting one well above background levels.

For photographic work the microfocus x-ray tube seems to be the simplest answer to the intensity problem since it is the brilliance of the focal spot which is important. However, if the necessity of accurate intensity measurements requires the use of geiger or proportional counters, then it will usually be found that the total flux from the focal spot is the important parameter and a rotating anode is indicated.

ONR Supported Publications

1. Multiple Diffuse Small Angle Scattering of X-Rays
David L. Dexter and W. W. Beeman
Phys. Rev. 76 1782 (1949)

2. Double Crystal and Slit Methods in Small Angle X-Ray Scattering
H. N. Ritland, P. Kaesberg and W. W. Beeman
Jour. of App. Phys. 21, 838 (1950)

3. An X-Ray Investigation of the Shapes and Hydrations of Several Protein Molecules in Solution
H. N. Ritland, Paul Kaesberg and W. W. Beeman
Jour. of Chem. Phys. 18, 1237 (1950)

4. Quantum Limits of the Electrostatic Image Force
R. G. Sachs and D. L. Dexter
Jour. of App. Phys. 21, 1304 (1950)

5. Applications of X-Ray Spectroscopy to Solid State Problems - Report of Madison Conference Oct. 23-25, 1950; Edited by W. W. Beeman, Published by the Physics Branch of the Office of Naval Research

6. Note on the Fluorescence Yield of Argon
D. L. Dexter and W. W. Beeman
Phys. Rev. 81, 456 (1951)

7. On the Size, Shape and Hydration of Southern Beam Mosaic Virus and Tobacco Necrosis Virus in Solution
B. R. Leonard, Jr., J. W. Anderegg, Paul Kaesberg, S. Shulman and W. W. Beeman
Jour. of Chem. Phys. 19, 793 (1951)

8. Note on the Absorption Spectra of Pure and Colored Alkali Halide Crystals
D. L. Dexter
Phys. Rev. 83, 435 (1951)

9. The Size of Latex Particles by X-Ray Scattering
B. R. Leonard, Jr., J. W. Anderegg, Paul Kaesberg and W. W. Beeman
Jour. of App. Phys. 23, 152 (1952)

10. The X-Ray Absorption Edges of Covalently Bonded Cr, Mm, Fe and Ni
G. Mitchell and W. W. Beeman
Jour. of Chem. Phys. 20, 1298 (1952)

11. Further Evidence Concerning the Periodic Structure in Collagen
Paul Kaesberg and M. M. Shurman
Biochimica and Biophysica Acta 11, 1 (1953)

12. An X-Ray Investigation of the Sizes and Hydrations of Three Spherical Virus Macromolecules in Solution.
B. R. Leonard, Jr., J. W. Anderegg, S. Shulman, Paul Kaesberg and W. W. Beeman
Biochimica and Biophysica Acta To be published late 1953

13. Small Angle X-Ray Scattering from Liquid Helium I and Liquid Helium II
A. G. Tweet
Phys. Rev. To be published early 1954

14. Small Angle X-Ray Scattering from Turnip Yellow Mosaic Virus
Paul Schmidt, Paul Kaesberg, and W. W. Beeman
Biochimica and Biophysica Acta
Submitted for publication

15. Small Angle X-Ray Studies of Bovine Fibrinogen
Paul Kaesberg, J. W. Anderegg and S. Shulman
Biochimica and Biophysica Acta
Manuscript to be submitted

16. Evidence on the Size and Shape of Serum Albumin Monomer and Dimer.
J. W. Anderegg, B. R. Leonard, Jr., S. Shulman, Paul Kaesberg and W. W. Beeman
Jour. Amer. Chem. Soc.
Manuscript to be submitted

17. Instrumentation for Small Angle X-Ray Scattering
W. S. Rothwell, B. R. Leonard, Jr., and W. W. Beeman
Reviews of Scientific Instruments
Manuscript in preparation

18. X-Ray Absorption Edges of Covalent Complexes of Elements of the First Transition Series
M. Shurman and W. W. Beeman
Jour. of Chem. Phys.
Manuscript in preparation

19. Pinhole and Slit Scattering Functions for Some Simple Shapes
Paul Schmidt, J. Anderegg and D. L. Dexter
Jour. of Applied Physics
Manuscript in preparation

20. Scattering from Bovine Serum Albumin and Bovine Hemoglobin in Pinhole Geometry.
W. S. Rothwell and W. W. Beeman
Jour. Amer. Chem. Soc.
Manuscript in preparation